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Regional changes in *c-fos* mRNA in rat brain after i.v. or i.c.v. NMDA injections

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THESE studies were undertaken to determine if convulsant doses of i.c.v. *vs* i.v. administered NMDA exhibit differential specificity for anatomical regions of the brain in stimulating *c-fos*. In rats i.c.v. or i.v. NMDA produced behaviorally similar clonic (popcorn) convulsions associated with transient increases in *c-fos* mRNA in different brain areas. Transcription of *c-fos* mRNA peaked at 30 min post-treatment regardless of the route of administration. However, the route of administration clearly influenced the anatomical specificity of the NMDA-induced *c-fos* mRNA changes. For example, following i.c.v. administration maximal stimulation in *c-fos* mRNA was measured in the cerebellum. In contrast, i.v. NMDA produced maximal *c-fos* mRNA stimulation in the cerebral cortex. Our results demonstrate that NMDA has differential anatomical specificity for molecular signaling in rat brain and suggest that the route of NMDA administration may influence its pathophysiological response.

Key words: NMDA receptor; *c-fos* mRNA; Brain regions; Convulsions; Route of administration

Introduction

In neuronal cells, activation of the prototypic immediate early gene *c-fos* has been reported following NMDA/glutamate receptor activation and depolarization.[1,2] These and other observations have led to the hypothesis that the *c-fos* oncogene product may be involved in the cellular changes responsible for the long term adaptation in the central nervous system. Studies from this laboratory have recently demonstrated that similar to parenteral (i.e. i.v.) administration, centrally (i.e. i.c.v.) administered NMDA produced severe clonic (popcorn) convulsions in rats.[3] However, unlike i.v. NMDA administration,[4] i.c.v. NMDA was *not lethal* and exhibited a unique sensitivity to various NMDA antagonists.[3] Therefore, using rat brain *c-fos* mRNA expression the present studies were undertaken to determine whether the route of administration differentially influences the pattern of regional brain responsiveness to NMDA, thereby providing a molecular tool to assess subsequent experimental (i.e. pathophysiological and pharmacological) responses.

Materials and Methods

Animals: Adult male Sprague–Dawley rats (Charles River; 200–250 g) naive to previous drug or seizure exposure were housed individually under controlled environmental and lighting (12 h light/dark cycle) conditions for at least one week, with food and water available *ad libitum*, before being used in experiments.

Drug administration: Rats were randomly divided into control or experimental groups. Three to five days before the experiment, rats were anesthetized with Ketamine (70 mg kg^{-1}, bw; Parke-Davis, Morris Plains, NJ) and Rompum (6 mg kg^{-1}, bw; Mobay Corp., Shawnee, KS) and surgically implanted with an i.c.v. cannula aimed at the right lateral ventricle and an i.v. external jugular vein catheter.[5,6] Control animals received either an i.v. (1 ml kg^{-1}, bw) or i.c.v. (5 μl) injection of saline and experimental animals received NMDA (12.5 nM, i.c.v. or 100 mg kg^{-1}, i.v.) and were sacrificed 15, 30, 60 or 120 min later. In additional experiments animals were treated as follows: vehicle only, or i.c.v. NMDA only, or i.c.v. NMDA + 1.9 μg, i.c.v. (+)MK-801; or i.v. NMDA only, or i.v. NMDA + 0.4 mg kg^{-1}, i.v. (+)MK-801. All animals were sacrificed 30 min later. In this series of studies (+)MK-801 was administered i.c.v. 15 min prior to, or i.v. 5 min prior to, NMDA.

Chemicals: NMDA and (+)MK-801 were purchased from Research Biochemicals Inc. (Natick, MA). All other chemicals were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO).

Tissue preparation and Northern blot analysis: Rats were sacrificed by decapitation, the brains quickly removed and the cerebral cortex, cerebellum and hippocampus were dissected out, frozen on dry ice, and stored at −70°C until further use. Total RNA was extracted using modifications[7] of a method described

Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulation relating to animals and experiments involving animals and adheres to the principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 85-23. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4–3), AR 360–5.

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by Cathala.[8] The Northern blots were generated and the amounts of *c-fos* mRNA were determined as described earlier.[7]

Results

As previously reported,[4] these doses of i.c.v. and i.v. NMDA consistently produced clonic (popcorn) convulsions in rats. I.c.v. injections of 12.5 nM NMDA resulted in popcorn convulsions in 33 of 40 rats tested, while all rats ($n = 52$) exhibited popcorn convulsions following i.v. NMDA administration. Only those animals exhibiting convulsions and surviving i.v. NMDA (64%) were included in the study.

Regardless of the route of administration, NMDA significantly induced *c-fos* mRNA in a time-dependent manner. However, the regional specificity of the *c-fos* activation was different depending upon the route of NMDA administration. The time course for changes in *c-fos* mRNA levels following i.c.v. NMDA administration is shown in Figure 1. Following i.c.v. administration of NMDA (Fig. 1A) the levels of *c-fos* mRNA in cerebellum were increased at 15 min, reached a maximal level at 30 min and dramatically declined to near control values by 60–120 min post-injection. The cerebral cortex exhibited a similar pattern of changes in *c-fos* mRNA levels. In contrast, *c-fos* mRNA in the hippocampus was only marginally (albeit not significantly) increased compared with control levels. Quantitative comparisons at 30 min after i.c.v. NMDA administration reveal a maximal increase in *c-fos* mRNA of approximately 5–8 fold in the cerebellum, followed by a 2–3 fold increase in the cerebral cortex while changes in the hippocampus were not significant.

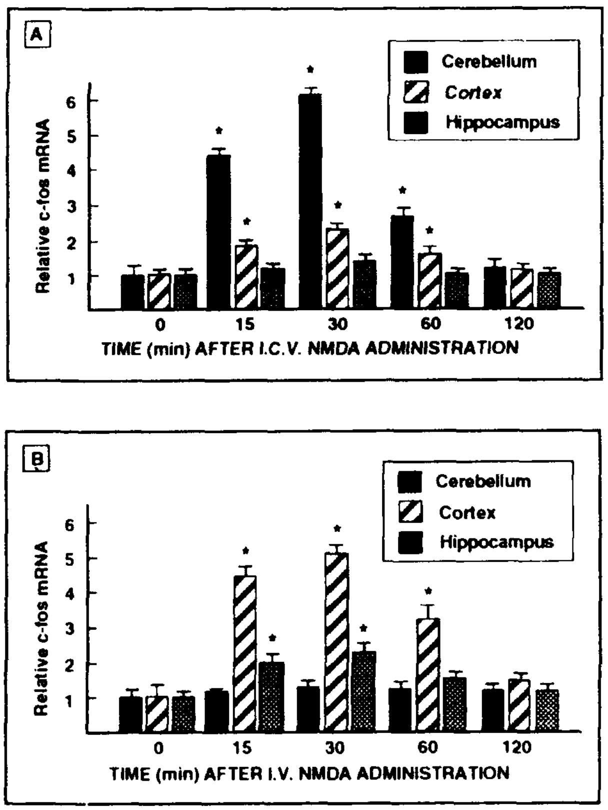

FIG. 1. Time course of changes in regional brain levels of *c-fos* mRNA in rats treated with i.c.v. NMDA (**A**) or i.v. NMDA (**B**). Values (mean ± standard deviation; $n = 3$) from control rats were set at 1 and values at different post-injection time intervals represent relative increases in *c-fos* mRNA over control values. This figure shows data from a representative experiment and was repeated with similar patterns of change. $^*p < 0.05$, at least, compared with controls (one factor ANOVA, Scheffe *F*-test).

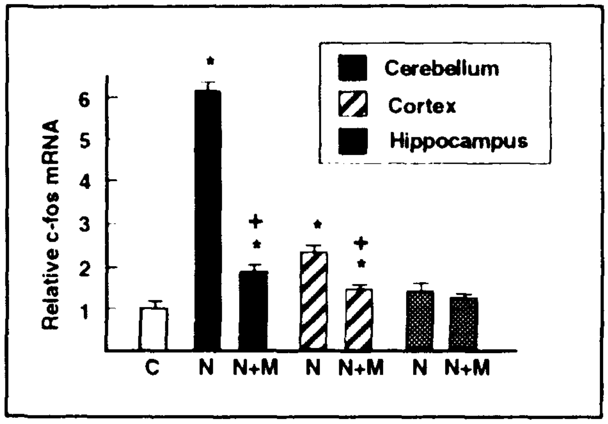

FIG. 2. Effects of 15 min pre-treatment with i.c.v. (+)MK-801 on *c-fos* mRNA 30 min after an i.c.v. NMDA injection. Values are the same as defined above for Figure 1. C = controls; N = NMDA administered group; N + M = NMDA and (+)MK-801 administered group. This figure shows data from a representative experiment and was repeated twice with similar patterns of change. $^*p < 0.05$, at least, compared with controls (Student's *t*-test). $^+p < 0.05$, at least, compared with NMDA alone.

Figure 1B shows the time course for changes in *c-fos* mRNA levels following i.v. NMDA administration. The levels of *c-fos* mRNA in the cerebral cortex were increased 15 min after NMDA administration, reached a maximal level 30 min after NMDA treatment and gradually declined to near control values within 120 min. Here, the hippocampus exhibited a similar temporal pattern of changes in *c-fos* mRNA levels. In contrast to i.c.v. NMDA, *c-fos* mRNA in the cerebellum was not significantly increased regardless of the time measured after i.v. NMDA. Similar to i.c.v. NMDA the maximal increase in *c-fos* mRNA was measured 30 min after NMDA administration regardless of the brain region studied. While the magnitude of the i.v. NMDA-induced *c-fos* was similar to i.c.v. NMDA, the pattern of change produced by i.v. NMDA was different. Approximately a 4–6 fold increase was observed in the cerebral cortex, followed by a 2–3 fold increase in the hippocampus while changes in the cerebellum were not significantly different from the control.

Administration of i.c.v. (1.9 μg) or i.v. (0.4 mg kg^{-1}) anticonvulsant doses of (+)MK-801 alone produced no significant changes in *c-fos* mRNA levels in either cerebellum, hippocampus or cerebral cortex (data not shown). However, when administered prior to i.c.v. NMDA or i.v. NMDA treatment, *c-fos* mRNA induction in these brain areas was significantly blocked by

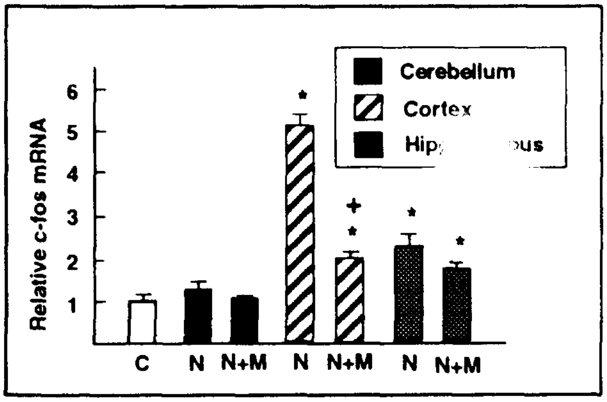

FIG. 3. Effects of 5 min pre-treatment with i.v. (+)MK-801 on *c-fos* mRNA 30 min after an i.v. NMDA injection. Values are the same as defined above for Figure 1. C = controls; N = NMDA administered group; N + M = NMDA and (+)MK-801 administered group. This figure shows data from a representative experiment and was repeated twice with similar patterns of change. *$p < 0.05$, at least, compared with controls (Student's *t*-test). +$p < 0.05$, at least, compared with NMDA alone.

i.c.v. or i.v. (+)MK-801, respectively (Figs 2 and 3). The greatest effect of i.c.v. (+)MK-801 on *c-fos* mRNA (Fig. 2) was observed in cerebellum where (+)MK-801 pretreatment resulted in approximately a 70% decrease in NMDA-induced *c-fos* mRNA. In the cerebral cortex *c-fos* mRNA was decreased only 40% by i.c.v. (+)MK-801 pretreatment while no significant effects were measured in the hippocampus. The greatest effect of i.v. (+)MK-801 on *c-fos* mRNA (Fig. 3) was observed in cerebral cortex where pretreatment with (+)MK-801 resulted in a 60% decrease in NMDA-induced *c-fos* mRNA. However, i.v. (+)MK-801 had no significant effect on either cerebellar or hippocampal *c-fos* mRNA changes produced by i.v. NMDA.

Discussion

The present study demonstrates that, similar to *in vitro* findings,[9,10] activation of the NMDA/glutamate receptor complex *in vivo* in rats is associated with an increase in the expression of the proto-oncogene *c-fos*. Regardless of the specific brain region studied or route of NMDA administration, the time-course analysis demonstrated that time to peak effect was 30 min post NMDA administration. The regional specificity in *c-fos* mRNA expression was dependent upon the route of NMDA administration. Following i.c.v. NMDA injections, the greatest increase in *c-fos* mRNA was measured in the cerebellum. In contrast, following i.v. NMDA administration the greatest increase was measured in the cerebral cortex. Pretreatment with the non-competitive NMDA antagonist (+)MK-801 significantly attenuated the NMDA-induced increase in *c-fos* mRNA levels in these brain areas.

The pattern of change in *c-fos* mRNA following non-NMDA seizures has been shown to be different from that observed in the present study following NMDA treatment in rats. For example, it was recently demonstrated that maximal electroshock convulsions in rats increased *c-fos* mRNA maximally in the cerebellum followed by the hippocampus and cerebral cortex.[7] However, in other studies, such as ethanol withdrawal-induced seizures in mice,[11] electroconvulsive shock in mice,[12] and by pentylenetetrazol,[13] kainic acid[14,15] or kindling[16] in rats a different regional pattern of *c-fos* mRNA, characterized by maximal expression in the hippocampus followed by the cerebral cortex and cerebellum, was measured. On the other hand, only a few studies have documented the activation of *c-fos* gene in brain following NMDA-induced seizures. In a recent *in situ* hybridization study, peripherally administered NMDA increased *c-fos* mRNA in the dentate gyrus of the hippocampus and piriform cortex and, similar to our results, did not produce significant changes in *c-fos* mRNA in the cerebellum.[17] In the present study we report a distinctively different pattern of *c-fos* mRNA expression which is dependent upon the route of NMDA administration and where maximal increases in *c-fos* mRNA were expressed in either the cerebellum or the cerebral cortex.

The pathophysiological importance of *c-fos* expression to epileptogenesis remains unclear, as does its possible role in the seizure protective mechanisms of anticonvulsant drugs.[7] Moreover, the functional consequences of abnormal *c-fos* expression to other neurophysiological conditions reflective of neuronal hyperexcitability and/or neurotoxicity awaits advanced investigations. However, recent *in vitro* studies from our laboratory showing that neuroprotection from glutamate toxicity can be achieved with *c-fos* antisense oligonucleotide have established a seminal role for the *c-fos* proto-oncogene as a critical signaling messenger mediating NMDA/glutamate receptor neuronotoxicity.[18] Since NMDA-induced transcription of *c-fos* mRNA appears to be a calcium requiring mechanism,[19] expression of the nuclear *c-fos* gene may represent a tertiary signaling mechanism linked to the pathological consequences of neuronal hyperexcitability.

While the precise mechanism(s) involved in this phenomenon remain to be elucidated, the observation in these studies of the lack of responsiveness of hippocampal *c-fos* mRNA to i.c.v. NMDA was surprising in view of the relative importance of this neocortical structure in mechanisms of seizure generation and control.[20] Furthermore, although both routes of NMDA administration produce nearly identical behavioral convulsions in rats, the present results confirm our earlier observation showing that only parenteral NMDA is lethal.[4] The lower incidence in lethality observed in this study may relate to differences in animal supplier (Charles River in the present study *vs* Zivic Miller in the earlier study[4]). While the physiological cause of NMDA-induced lethality in these rodents has not been identified, our own observations indicate

that the animals become severely apneic shortly following the convulsion, usually dying within 5–10 min of the i.v. NMDA injection. While the results of the present study may fail to implicate a critical role for the hippocampus in NMDA convulsions, they appear to suggest that the immediate transcription of *c-fos* oncogene in the hippocampus may be functionally involved in the lethality associated with parenteral NMDA administration.

Conclusions

The results of the present study demonstrate that centrally or peripherally administered NMDA in rats induces the expression of *c-fos* proto-oncogene in a pattern distinct from other experimental seizures. Importantly, there was a direct correlation between the pattern of seizure-induced *c-fos* expression and the site-specific inhibitory effects of the anticonvulsant drug MK-801. Since the oncogene *c-fos* appears to play an important role in long-term plasticity associated with a number of chronic NMDA/glutamate mediated CNS pathologies, including epilepsy, stroke, neurodegenerative diseases and related learning/memory dysfunctions, we suggest that proto-oncogene expression may have important physiological and/or pathological implications for understanding the molecular mechanism(s) of NMDA-related CNS dysfunction and the related development of improved treatment strategies.

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